Re-Engineering The Bacteriophage Coat for Avid Display of Fibronectin Domains

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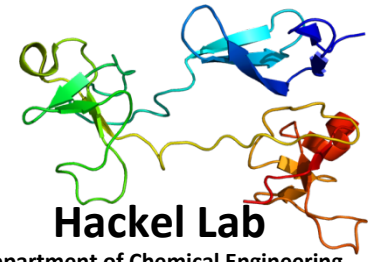

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ABSTRACT

Phage display is a compelling technique of synthetic binding protein engineering which allows for a firm genotype-phenotype linkage which can be applied to selection and evolution of engineered binding protein scaffolds. Mutations can be made to the pVIII protein providing capability to significantly increase the surface display of large proteins on a phage particle (1) . Random and rational mutagenesis of the major coat protein, pVIII, was performed on the first 30 residues in 10-mer segments. The array of mutations imbedded into the wild type pVIII was mass produced to construct a phage display library. Promising mutants will selected by the screening of avid activity by ELISA selection. The effective clones were paired with DNA from the fibronectin Gr2 library, and captured on ELISA plates by binding interaction with hen egg lysozyme for quantification by plate reader. Initial experiments yielded no avid clones, but this is likely due to the weak affinity of the first Fn chosen for capture ELISA in these experiments. Adjustments were made and are being tested to discover pVIII clones with avid functionality.

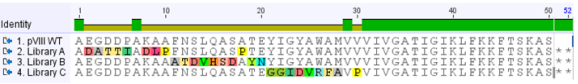

Figure 1: Mutations were made to the first 30 residues of wild type pVIII in successive 10-mer segments. An overlay of three mutant library clones shows mutations were successful.

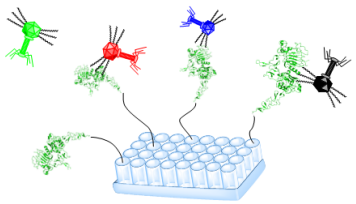

Diagram 1: General diagram of cell panning shows how multivalent expression of pVIII-fusion protein avidly interact with target antigen.

METHODS

Wild type pVIII was constructed with assembly PCR. Mutations were implemented through introduction of mutagenic degenerate oligonucleotides and introduced into a phage display vector containing low affinity lysozyme-binding fibronectin (Fn) clones (2) . The Fn-pVIII fusion mutant libraries were transformed into XL1-Blue E. Coli, along with helper phage to mass produce phage libraries.

Phage libraries with mutant expression were sorted by ELISA plate for detection of avid activity. Promising clones were taken through ELISA screening in comparison to naïve Fn clones for quantification. In future studies the clones expressing favorable avidity will be utilized for detection of target interactions on tumor cell lines.

DATA & RESULTS

Background Corrected

	1	2	3	4	5	6
A	1.695	1.802	0.02	1.837	-0.029	-0.01
B	0.547	0.259	0.101	1.816	1.99	0.114
C	-0.111	0.57	0.011	1.905	2	2.245
D	2.134	0.115	0.027	1.497	1.843	-0.113
E	0.933	-0.057	0.017	0.23	-0.007	2.245
F	1.423	1.978	0.481	1.52	1.637	0.004
G	1.997	0.16	0.204	1.514	-0.097	-0.012
H	1.858	-0.008	-0.029	2.205	-0.001	1.42

Figure 3: 0.3b sub-library clones were selected by ELISA sorting and quantified by fluorescent plate reader. Signals from positive antigen wells were normalized to background with negative antigen wells. Normalized signals less than 1 but greater than 0 (light green) were brought through another round of selection.

	1	2
A	0.084	-0.066
B	0.66	-0.08
C	1.426	0.132
D	0.312	0.008
E	-0.021	0.605
F	1.752	0.013
G	0.282	0.02
H	0.134	3.243

Figure 4: Clones of interest selected in **Figure 2** were taken through a second round of ELISA screening. When normalized over background, clones 1B, IC, ID, 2C and 2E were sequenced. Red is two negative (pAKDM RDG [F2], L0.7.1 [G2]) and one positive control (GaL 0.3b.4 [H2])

DISCUSSION

Mutations into the first 30 residues of pVIII in 10-mer segments were readily accepted (**Figure 1**). Initial selections from the pVIII libraries using a very weakly lysozyme-binding Fn clone did not yield initial hits from any library. It is our hypothesis that this Fn clone was not a strong enough binder to be recovered, even with avid interaction. To remedy this, several new Fn clones from the Gr2 library were tested by ELISA to find a ligand for use in these studies. Two rounds of ELISA binding experiments were carried out to assess binding ability and several clones were brought forward as candidates (**Figures 2 and 3**). The future pVIII clone selection experiment has also been optimized for better detection of avid binding interactions. These avid binding interactions will be extremely important in later experiments with applications to cancer biomarker discovery

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1.) Sidhu, Sachdev S., Gregory A. Weiss, and James A. Wells. "High Copy Display of Large Proteins on Phage for Functional Selections." Journal of Molecular Biology 296.2 (2000): 487-95. Web.
2.) Benjamin J. Hackel, Atul Kapila, K. Dane Wittrup, Picomolar Affinity Fibronectin Domains Engineered Utilizing Loop Length Diversity, Recursive Mutagenesis, and Loop Shuffling, Journal of Molecular Biology, Volume 381, Issue 5, 19 September 2008, Pages 1238-1252